Ultrastructural study of oogenesis in the African mussel, *Perna perna* (Bivalvia: Mytilidae)

Estudio ultraestructural de la ovogénesis en el mejillón africano, *Perna perna* (Bivalvia: Mytilidae)

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ABSTRACT

Transmission electron microscopy of female gonads in *Perna perna* (Mytilidae) was carried out to study the fine structure of the different developmental stages during oogenesis and to examine the functions of the follicular cells. The gonad is composed of acini, each containing oogoniae and oocytes surrounded by follicular cells, and of an interacinar connective tissue with vesicular cells. Groups of primary oogoniae, surmounted by secondary oogoniae, were found along the inner wall of each acinus. Previtellogenesis of oocytes was characterized by a great increase of these cells in volume, the accumulation of numerous organelles, and the formation of the first yolk granules at the end of this phase. The period of vitellogenesis involved both autosynthetic and heterosynthetic pathways, and was marked by the accumulation of cortical granules and of yolk granules. Numerous lipid droplets (two types), several inclusions resulting from the heterosynthetic uptake of exogenous substances by pinocytosis, and droplets showing two types of electron-lucent materials in the cytoplasm of mature oocytes could also be observed. In the atretic ovocytes, a vacuolization progressively developed in their cytoplasm, with the subsequent rupture of the vitelline membrane and the release of oocyte remnants in the lumen of the acinus. At the end of vitellogenesis, the follicular cells became detached from the oocyte and contained numerous lipid droplets and glycogen inclusions. In *P. perna*, the formation of female gametes was mostly similar to the oogenesis described in other species of bivalvia. The only difference concerned the composition of the yolk in the mature oocyte, as it was constituted of several endogenous substances (lipoproteins mainly) and also of exogenous materials intaken by the oocyte via pinocytosis.

RESUMEN

Las gónadas femeninas en *Perna perna* (Mytilidae) se observaron por microscopía electrónica de transmisión para estudiar la estructura fina de las diferentes etapas de desarrollo durante la ovogénesis y examinar las funciones de las células foliculares. La gónada está formada por acinos, cada uno de ellos conteniendo oogonias y ovocitos rodeados por células foliculares, y por un tejido conectivo interacinar con células vesiculares. Grupos de oogonias primarias, a los que se sobreponen oogonias secundarias, se encuentran a lo largo de la pared interna de cada acino. La previtelogénesis de los ovocitos se caracterizó por un gran aumento de volumen en estas células, por la acumulación de

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numerosos orgánulos y por la formación de los primeros gránulos de vitelo al final de esta fase. El período de vitelogénesis implica ambas vías autosintética y heterosintética y se caracteriza por la acumulación de gránulos corticales y de los gránulos de vitelo. En el citoplasma de los ovocitos maduros, se observaron también numerosas gotitas de lípidos (de dos tipos), varias inclusiones resultando de la absorción heterosintética de sustancias exógenas por pinocitosis, así como gotitas con dos tipos de materiales electrón lúcidas. En los ovocitos atrésicos, una vacuolización se desarrolló progresivamente en su citoplasma, con la posterior ruptura de la membrana vitelina y la liberación de los restos de ovocitos en el lumen de los acinos. Al final de la vitelogénesis, las células foliculares se desprendían del ovocito y contenían numerosas gotitas de lípidos y inclusiones de glucógeno. En *P. perna*, la formación de gametos femeninos fue muy similar a la ovogénesis descrita en otras especies de bivalvos. La única diferencia se refiere a la composición del vitelo en el ovocito maduro, ya que está constituido por varias sustancias endógenas (lipoproteínas, principalmente), así como por materiales exógenos absorbidos por el ovocito, mediante pinocitosis.

INTRODUCTION

The African mussel: *Perna perna*, is a worldwide species (Berry, 1978; Hicks , Tunnell and McMahon, 2001a). However, the studies performed on the biology of this mussel only concerned its reproductive cycles, as there was an intraspecific variability in the reproduction. Indeed, in the South African populations of *P. perna*, two main spawning periods between April and October, and several minor and scarcer spawning events after October were reported by Berry (1978) and Lasiak (1986). By contrast, in other *Perna* populations from the same country, Schurink and Griffiths (1991) found a single prolonged spawning period along the year, with spawning activity every month. In the Gulf of Mexico, three spawning periods with one extended and two discrete events in spring or summer were described by Hicks, Tunnell and McMahon (2001) and Hicks, McMahon and Ingrao (2001). In view of this variability in reproductive cycles, it was useful to study gametogenesis in local populations of *P. perna*.

As *P. perna* lives along the southern Atlantic coasts of Morocco, a research programme was carried out to determine the spawning episodes of these local mussels and to analyse the characteristics of their gametogenesis. A first histological study (Id Halla, Bouhaimi, Zekhnini, Narbonne, Mathieu and Moukrim, 1997) demonstrated a single spawning period along the year, with a major event in spring, and this work was completed by ultrastructural studies on spermatogenesis in the same Moroccan population of *P. perna* (Benomar, Belhsen, Goux, Mathieu and Moukrim, 2007). The main aim of the present paper is to describe the ultrastructural stages of female gamete formation and of oocyte degeneration in *P. perna*. The follicular cells and their function are also examined.

MATERIALS AND METHODS

Samples of five mussels each (3-4 cm long) were collected in December 1999, January, March, June and July 2000 from the mid-tide level at Cap Ghir (50 km north of Agadir town). Small portions of female gonad (1-3 mm^3 each) were fixed for 60 min in 2% glutaraldehyde (0.4 M sodium cacodylate buffer, pH 7.2) at 4°C. The tissue was then washed in 0.4 M cacodylate buffer (3 x 10 min) and postfixed for 90 min in 1% osmium tetroxyde (in 0.4 M cacodylate buffer) at 4°C. After dehydration through a gradual ethanol series, the tissue was directly embedded in Epon resin at 37 °C for 60 min and was subsequently

Figure 1. A. Primary oogonia (Og) and follicular cells (Fc) of *Perna perna*, along the inner side of the acinar wall. Some vesicular cells (Vc) were located along the outer side. da, dense aggregates; G, Golgi apparatus; gl, glycogen; Li, lipid droplets; m, mitochondria; N, nucleus, RER, rough endoplasmic reticulum; pc, cytoplasmic projections of follicular cells; v, vacuoles. B. Interacinar vesicular cells showing two types of granules (g1, and g2). Scale bars, 2 µm.

Figura 1. A. Oogonia primaria (Og) y células foliculares (Fc) de Perna perna, *sobre el lado interno de la pared acinar. Algunas células vesiculares (Vc) se ubicaron en el lado externo. da, agregados densos; G, Aparato de Golgi; gl, glucógeno; Li, gotitas de lípidos; m, mitocondria; N, núcleo, RER, retículo endoplasmático rugoso; pc, proyecciones citoplasmáticas de células foliculares; v, vacuolas. B. Células vesiculares interacinares mostrando dos tipos de gránulos (g1, and g2). Escalas, 2 µm.*

placed at room temperature during the following 12 hours. Semi-thin sections of each gonad portion were stained at room temperature with 0.5% toluidine blue in 2.5% Na_2CO_3. Ultrathin sections were collected on copper grids and were stained for 20 min with uranyl acetate, followed by lead citrate for 5 min. Sections were examined using a Siemens 102 electron microscope.

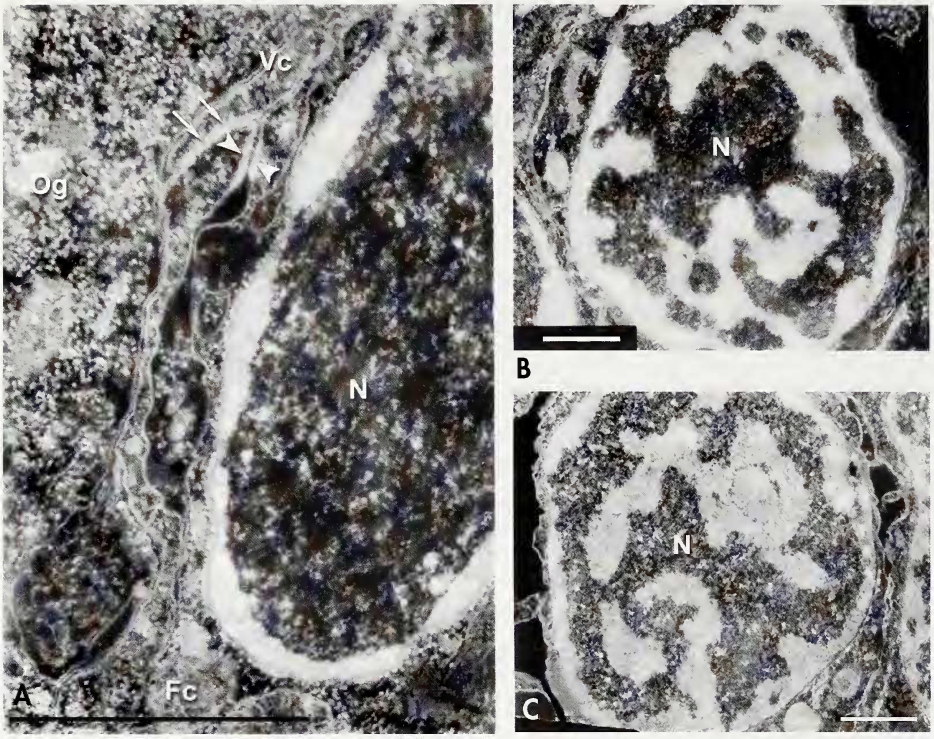

Figure 2. Oogoniae of *Perna perna*. A. Zonula occludens (arrows) and zonula adherens (arrow heads) between an oogonia (Og), a follicular cell, and a spindle-shaped cell. B-C. Nuclei of two oogoniae in metaphasis (first meiotic division). Scale bars, 2 µm.
Figura 2. Oogonias de Perna perna. *A. Zonula occludens (flechas) y zonula adherens (puntas de flecha) entre una oogonia (Og), una célula folicular, y una célula fusiforme. B-C. Núcleos de dos oogonias en metáfase (primera división meiótica). Escalas, 2 µm.*

Different measurements for each cell stage of ovogenesis (a least of 10 cells per stage) were also performed. Individual values recorded for each measurement and each cell stage were averaged.

RESULTS

Numerous acini, surrounded by connective tissue and an external ciliated epithelium, constituted each gonad (Fig. 1A). Along the inner side of each acinar wall, the different developmental stages of oogenesis, from oogoniae up to the first stages of the first meiotic division, could be easily observed. All of them were surrounded by follicular cells and spindle-shaped cells containing glycogen inclusions (Fig. 1A). In the interacinar connective tissue, other vesicular cells, each containing two types of granules, were found (Fig.1B). Along the outer side of the acinus, several muscular fibers were sometimes observed (Fig. 3A).

Oogoniae: Groups of primary oogoniae (primordial cells), each constituted by 2 or 4 cells, were found along the inner side of each acinar wall (Fig. 1A, B). Follicular cells characterized by their cytoplasmic extensions were close to these cell groups. The primary oogoniae were round or elongated, measured 5 to 8 mm in size, and were characterized by a high nucleo-cytoplasmic ratio. They were interconnected by zonulae occludens and zonulae adherens (Fig. 2A). Their nuclei (3-6 µm) contained small

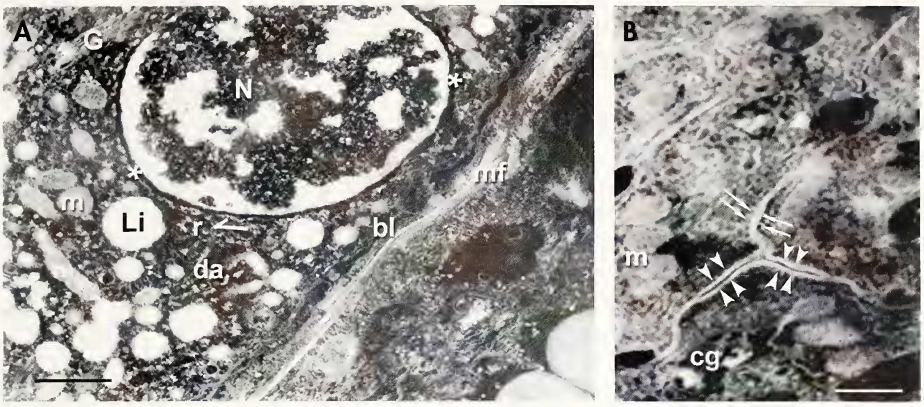

Figure 3. Previtellogenic oocyte of *Perna perna*. A. Early previtellogenic oocyte. The nucleus (N) showed a wide perinuclear cisterna (*) and is positioned near the basal lamina (bl) of the acinus. G, Golgi apparatus; Li, lipid droplets; m, elongated mitochondria; mf, muscular fibers; r, ribosomes. B. Late previtellogenic oocyte. Zonula occludens (double arrows) and zonula adherens (arrow heads) between one oocyte and two follicular cells. Scale bars, A: 1 µm; B: 0.2 µm.
Figura 3. Ovocitos previtelogénicos de Perna perna. *A. Ovocito previtelogénico temprano. El núcleo (N) presentaba una cisterna perinuclear (*) ancha y se sitúa cerca de la lámina basal (bl) del acino. G, Aparato de Golgi; Li, gotitas de lípidos; m, mitocóndria alargada; mf, fibras musculares; r, ribosomas. B. Ovocito previtelogénico tardío. Zonula occludens (flechas dobles) y zonula adherens (puntas de flecha) entre un ovocito y dos células foliculares. Escalas, A: 1 µm; B: 0,2 µm.*

patches of chromatin. In several cells, the chromatin formed a thin border along the inner side of the nuclear envelope, a single or two nucleoli could be observed, and the nuclear envelope was indented. Numerous ribosomes, spherical mitochondria, some cisternae of endoplasmic reticulum, several lipid droplets with no membrane, and membrane-bounded dense vesicles were found in the cytoplasm (Figs. 1-2).

The secondary oogoniae measured 5 to 6 µm in size, and the diplotene stages of the first meiotic division (Fig. 2B, C) were easily recognizable in their voluminous nuclei (4.5-5.5 µm). However, synaptonemal complexes were not found in these nuclei. These cells contained the same organelles as described for primary oogoniae.

Previtellogenic oocytes: The early previtellogenic oocytes (Fig. 3A) were connected together and to follicular cells via zonulae occludens and zonulae adherens (Fig. 3B). They were round or elongated, with irregular outlines, and their size ranged from 9 to 15 µm. Their cytoplasm was more voluminous than that of oogoniae and was increased by the addition of ribosomes, of often elongated mitochondria, and of smooth or rough endoplasmic reticulum (ER). Several vacuoles around the Golgi apparatus could be observed. Their nucleus became elongated, with scattered chromatin and a thicker nuclear envelope, showing a clearly wider perinuclear cisterna and the presence of ribosomes along the outer side of the outer nuclear membrane.

At a later stage (Fig. 4A-E), the oocytes were elongated and their size reached up to 18 µm. Their nuclei became irregular, multilobulated, and were eccentrically positioned. Numerous pores through both membranes of the nuclear envelope could be seen and the nucleolus was often in an eccentric position. At the same time, dense aggregates with no membrane appeared in the cytoplasm, near the nucleus. The rough ER increased in size and some

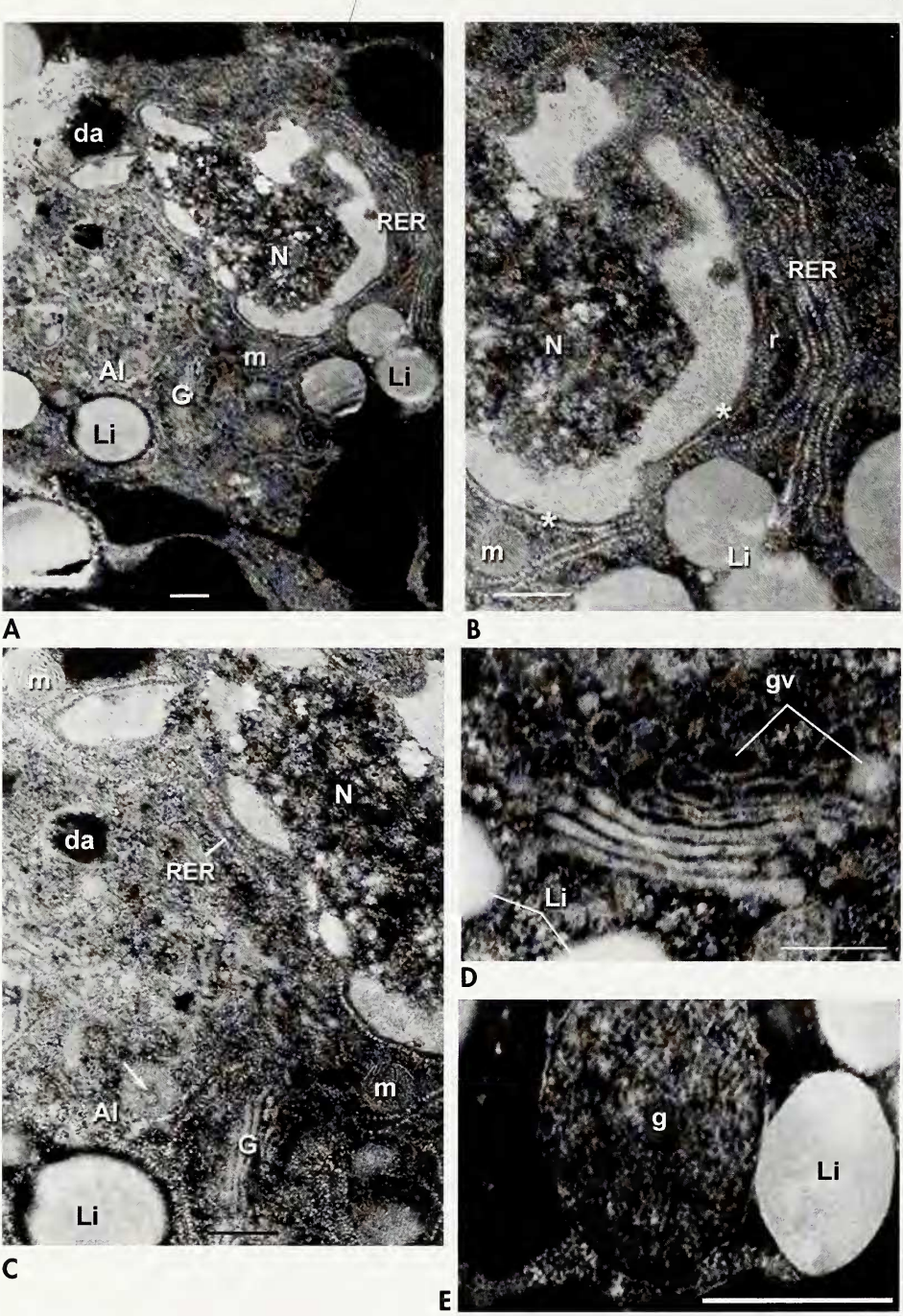

Figure 4 A-E. Late previtellogenic oocytes of *Perna perna*. Al, annulate lamellae; da, dense aggregates; G, Golgi apparatus with a vesicle (gv): g, granule; Li, lipid droplets; m, mitochondria; N, nucleus; RER, rough endoplasmic reticulum; arrow, yolk granule. Scale bars, 1 µm.

Figura 4 A-E. Ovocitos previtelogénicos tardíos de Perna perna. *Al, laminillas anulares; da, agregados densos; G, Aparato de Golgi con una vesícula (gv): g, gránulo; Li, gotitas de lipidos; m, mitocóndria; N, núcleo; RER, retículo endoplasmático rugoso; flecha, gránulo de vitelo. Escalas, 1 µm.*

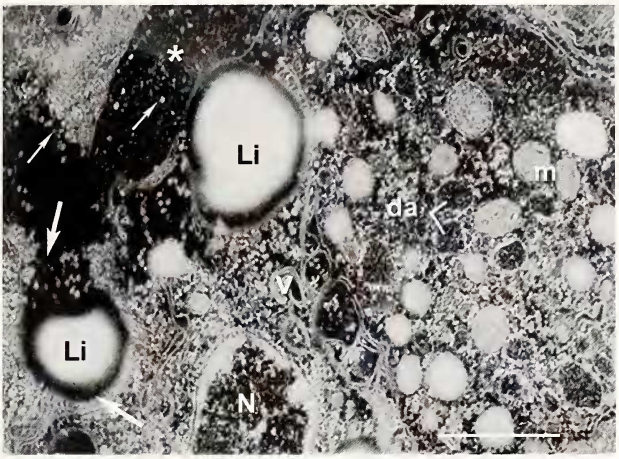

Figure 5. Pinocytosis vesicle (large arrow) with a lipid droplet and glycogen particles (small arrows). *, glycogen deposit in a spindle-shaped cell. Zonula occludens (double arrows) and zonula adherens (arrow heads) between one oocyte and two follicular cells. Scale bar, 1 µm.
*Figure 5. Vesícula de pinocitosis (flecha grande) con una gotita de lípidos y partículas de glucógeno (flechas pequeñas). *, depósito de glucógeno en una célula fusiforme. Zonula occludens (flechas dobles) y zonula adherens (puntas de flecha) entre un ovocito y dos células foliculares. Escala, 1 µm.*

lipid vesicles became bigger in its vicinity. In close proximity, elongated granules containing a dense granular material were sometimes observed. Lamellar structures of ER and the first yolk granules appeared near the Golgi apparatus (Fig 4C). In the basal part of the ooplasm and particularly in the zones of contact between the oocyte and its surrounding cells (follicular cells, and spindle-shaped cells), some pinocytosis vacuoles, each containing lipid droplets and several deposits probably of glycogen, could be seen (Fig. 5).

Vitellogenic oocytes: Their development within the gonad can be arbitrarily divided into four stages, from A the youngest, to D the oldest. Each early vitellogenic oocyte (A stage) kept contact with the acinar wall by cytoplasmic projections (Fig. 6A, B) and was surrounded by follicular cells which are apically connected by desmosome-like junctions. Measuring 30 µm in size, this oocyte contained an elongated nucleus (9 µm) with a very dense nucleolus. In the zone of attachment of the oocyte with the acinar wall, and particularly in cytoplasmic projections, some cisternae of rough ER could be observed. Several microvilli appeared on the outer surface of the apical oolemma (Fig. 6A). At a later stage in vitellogenesis (B stage), the nucleus became spherical and increased in size (Fig. 6C). The nucleolus was in an eccentric position and was surrounded by an electron-lucent material, while the nucleoplasm was constituted by moderately dense patches of heterochromatin and scattering euchromatin. A previtelline space was forming between the apex of the oocyte and the surrounding follicular cells, and an amorphous electron-lucent material, originating from the oocyte, settled into this space between the numerous apical microvilli formed by the oolemma. Progressively, this previtelline space extended along the lateral sides of the oocyte and the microvilli became coated by the amorphous material. Concurrently, there was an increase in the number of saccules forming the Golgi apparatus and in the number of mitochondria (mainly in the attachment zone of the oocyte) (Fig. 6A). The rough ER greatly increased in volume and showed circular lamellae, surrounded by numer-

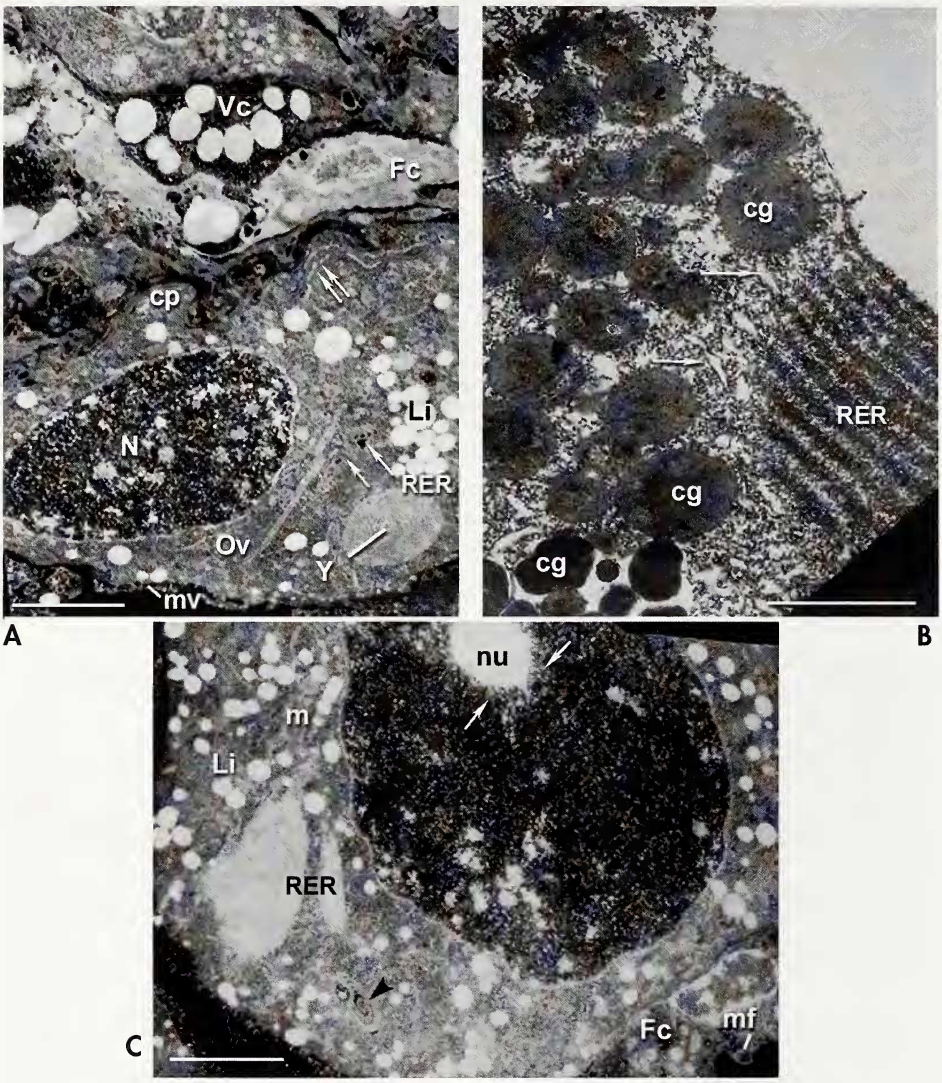

Figure 6. Vitellogenic oocytes of *Perna perna*. A. A-stage oocytes, with cytoplasmic projections (cp) and microvilli (mv) developing at the apex of cells. Flattened rough ER cisternae (RER) were positioned near the nucleus or in cytoplasmic projections (cp). A ring of rough ER surrounded a yolk granule (Y). Li, lipid droplets; arrow head, a Balbiani's body. B. cortical granules (cg) near several cisternae of rough ER (arrows). C. B-stage oocytes. Their nucleolus (nu) was in an eccentric position and was surrounded by an electron-lucent nuclear material (arrows). A myelin-like figure (mf) in a follicular cell can be observed. Flattened cisternae of rough ER were located in the vicinity of the nucleus. Scale bars, A, C: 2 µm; B: 1 µm.

Figura 6. Ovocitos vitelogénicos de Perna perna. *A. Ovocitos de estadio A, con proyecciones citoplasmáticas (cp) y microvellosidades (mv) desarrollandose en el ápice de las células. Cisternas aplanadas del RE rugoso (RER) eran situadas cerca del núcleo o en proyecciones citoplasmáticas (cp). Un anillo de RE rugoso rodeaba un gránulo de vitelo (Y). Li, gottas de lípidos; punta de flecha, un cuerpo de Balbiani. B. gránulos corticales (cg) cerca de varias cisternas de RE rugoso (flechas). C. Ovocitos de estadio B. Su nucleolo se situaba en posición excéntrica y era rodeado por un material electrón lúcido del núcleo (flechas). Se puede observar una figura parecida con mielina (mf) en una célula folicular. Algunas cisternas aplanadas de RE rugoso se situaban en las inmediaciones del núcleo. Escalas, A, C: 2 µm; B: 1 µm.*

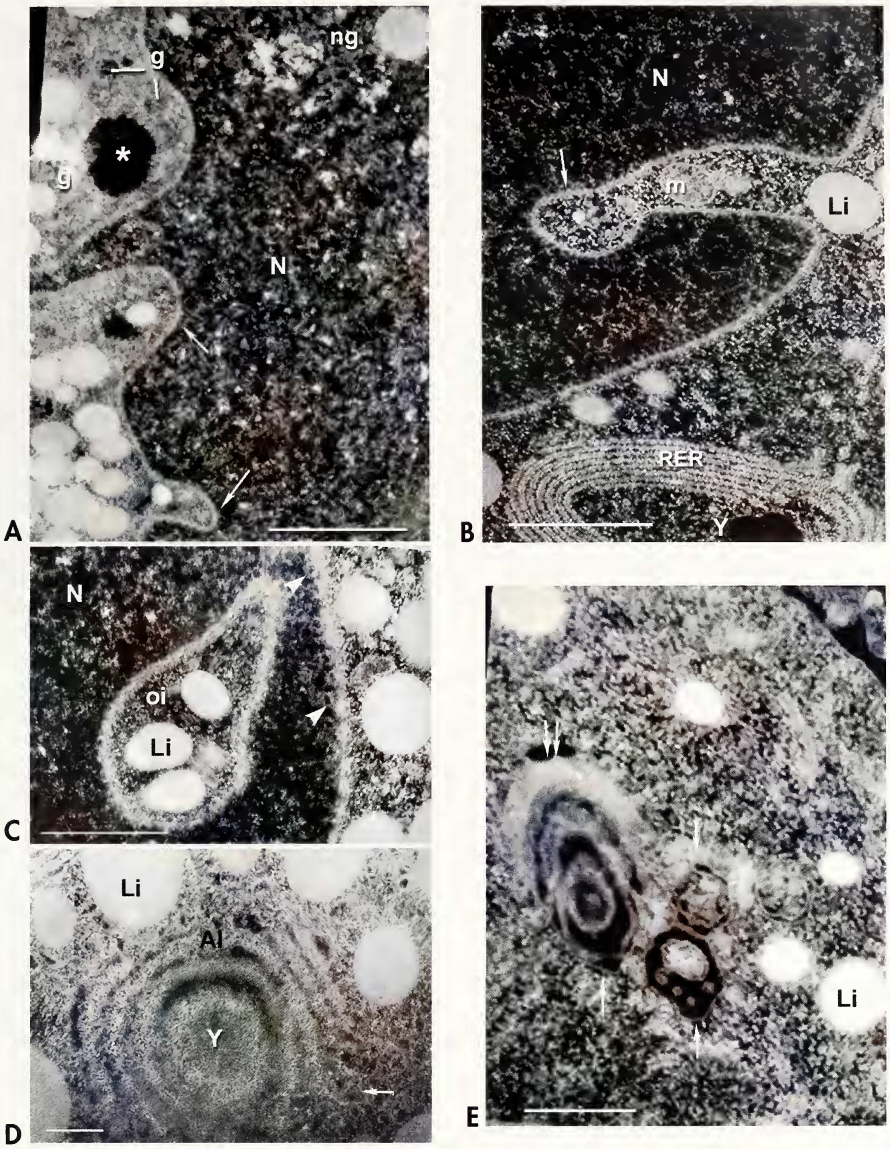

Figure 7. Vitellogenic oocytes of *Perna perna*. A-E. C-stage oocytes showing cytoplasmic indentations (oi, or arrows) in their nuclei. g, granules located in the ooplasm; Li, lipid droplets; *, a dense granule with no membrane. See also an electron-lucent patch (ng) in the nucleus (in A), a yolk granule (Y) surrounded by a ring of rough ER (in B), the presence of numerous pores (arrow heads) through the nuclear envelope (in C), that of annulate lamellae (Al) surrounding a yolk granule (in D), and Balbiani's vitelline bodies (arrows) near cisternae of rough ER (double arrow) (in E). Scale bars, A, B: 5 µm; C-E: 1 µm.

Figura 7. Ovocitos vitelogénicos de Perna perna. *A-E. Ovocitos de estadio Cmostrando indentaciones citoplasmáticas (oi, o flechas) en sus nucleos. g, gránulos situados en el ooplasma; Li, gotitas de lipidos; *, a gránulo densosin membrana. Nótese también una mancha electron lúcida (ng) en el núcleo (en A), un gránulo de vitelo (Y) rodeado por un anillo de RE rugoso (en B), la presencia de numerosos poros (puntas de flecha) atravesando la membrana nuclear (en C), la de laminillas anulares (Al) rodeando un gránulo de vitelo (en D), así como los cuerpos vitelinos de Balbiani (flechas) cerca de cisternas del RE rugoso (flechas dobles) (en E). Escalas, A, B: 5 µm; C-E: 1 µm.*

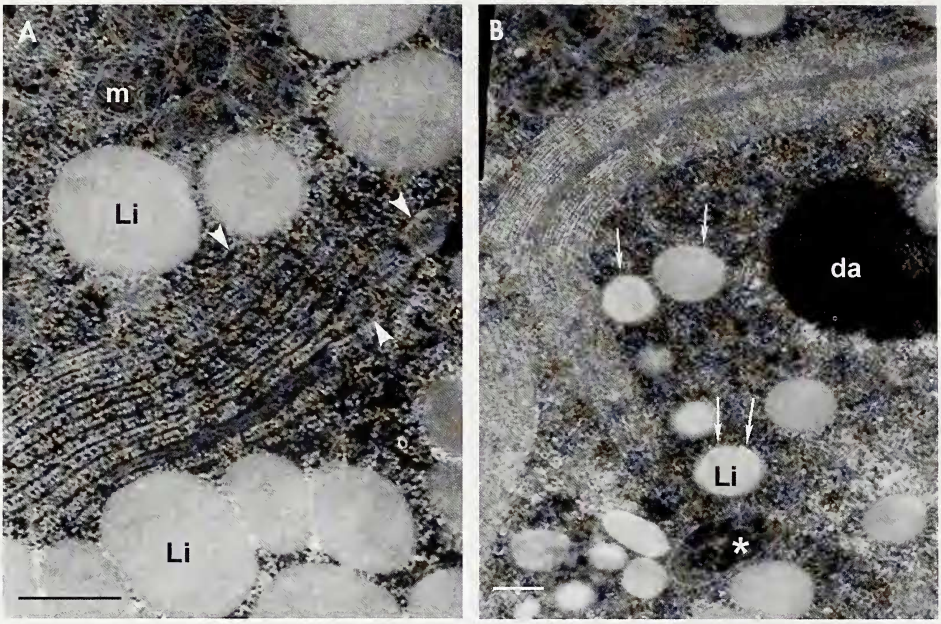

Figure 8. Vitellogenic oocytes (D stage) of *Perna perna*. A-B. Different shapes of the rough ER, with a proliferation of lipid droplets near mitochondria (m) and rough ER cisternae (arrow heads). da, dense granules; arrows, glycogen particles linked to lipid droplets; *, cortical granules (in B). Scale bars, 1 µm.

Figura 8. Ovocitos vitelogénicos (estadio D) de Perna perna. *A-B. Distintas formas del RE rugoso, con proliferación de gotitas de lípidos cerca de una mitocóndria (m) y cisternas del RE rugoso (puntas de flecha). da, gránulos densos; flechas, partículas de glucógeno ligadas a gotitas de lípido; *, gránulos corticales (en B). Escalas, 1 µm.*

ous lipid vesicles. Some cortical granules (Fig. 6B) limited by an undulating membrane, each containing a thin granular material (probably of glycoproteinic origin), were also observed near the rough ER and the Golgi apparatus. They are formed by an autosynthetic way from these organelles. They became progressively denser and were localized at the periphery of the oocyte.

The mature oocytes (C stage) reached 70 mm in size (Fig. 7A-E), while their shape became pedunculate. They were connected to follicular cells by zonulae occludens and zonulae adherens. In their nuclei (30 mm), the ring- or crescent-shaped nucleolus was dense and in an eccentric position, while numerous pores through the nuclear envelope could be observed. Deep cytoplasmic indentations (Fig. 7A-C) could be seen in the nucleus, each containing membrane-bounded lipid droplets and other dense granules with no membrane. The microvilli and the amorphous material constituted a vitelline membrane, which became detached later from the oocyte and thus created a dense perivitelline space between the oocyte and the vitelline membrane. The lamellae of rough ER (Figs. 6-8) were of variable forms (annulate, circular, ovoid, flattened, or crescent-shaped) and were often positioned near the nucleus. The C oocytes contained the same organelles as described for B cells. However, four other structures, often positioned in the vicinity of the rough ER and mitochondria, could be noted. First, membrane-bound lipid vesicles (0.8 mm) became larger via their fusion (Fig. 9A). Secondly, other lipid inclusions (0.5 mm)

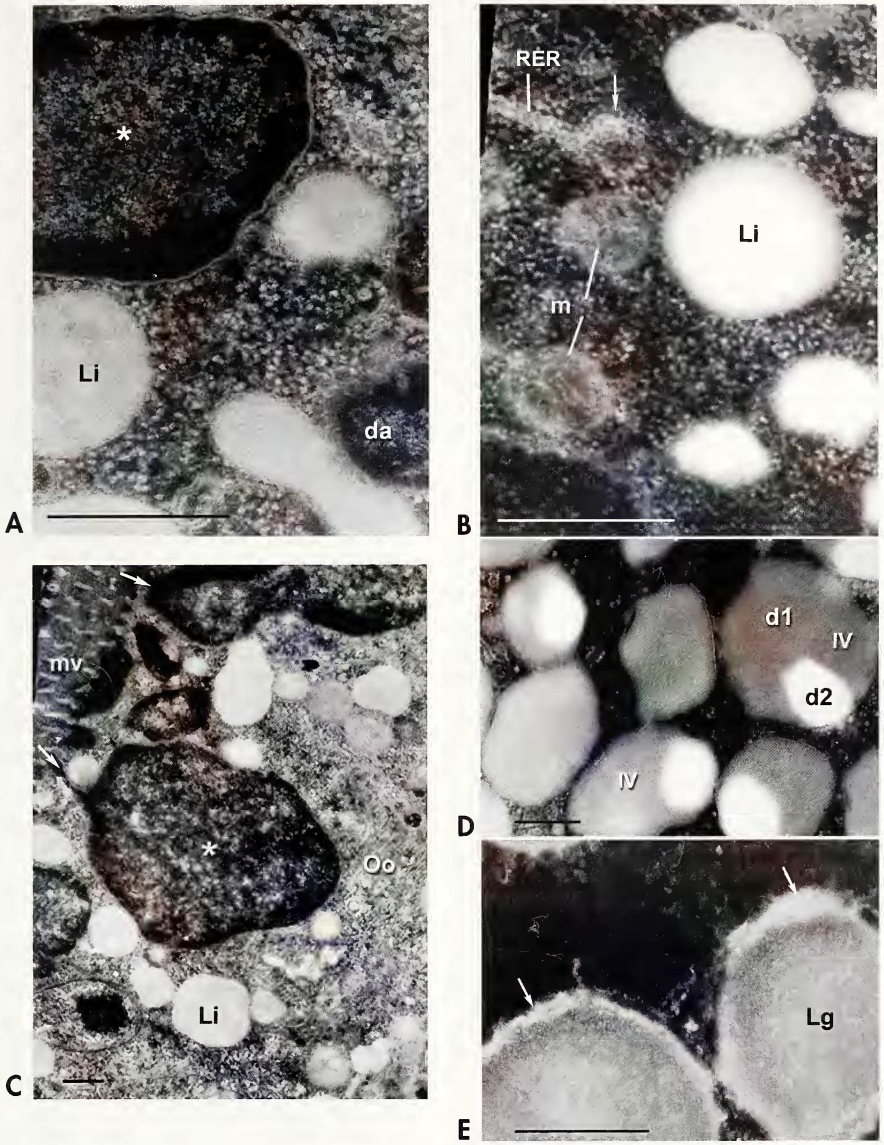

Figure 9 A-E. Vitellogenic oocytes (D stage) of *Perna perna*, different types of granules. See the single membrane-bounded lipid (Li) droplets (in A), several lipid inclusions with no membrane, near mitochondria (m) and cisternae of rough ER (in B), some membrane-bounded inclusions containing a granular material (*) and resulting from pinocytosis at the periphery of the ooplasm (arrow) (in A and C), several electron-lucent granules (IV), each showing two zones of different densities (d1, and d2) (in D), lipid droplets, each linked to a particle of glycogen (arrows) (in E, see also Fig. 8B). Scale bars, 1 µm.

Figura 9 A-E.. Ovocitos vitelogénicos (estadio D) de Perna perna, *distintos tipos de gránulos. Nótense las gotitas de lípidos delimitadas por una membrana sencilla (Li) (en A), varias inclusiones de lípidos sin membrana, cerca de mitocondrias (m) y cisternas de RE rugoso (en B), algunas inclusiones delimitadas por membranas, conteniendo material granular (*) y resultando de pinocitosis a la periferia del ooplasma (flecha) (en A y C), varios gránulos electrón lucidos (IV), cada uno con dos zonas de densidad diferente (d1 y d2) (in D), gotitas de lípidos, cada uno ligado a una partícula de glucógeno (flechas) (en E, véase también Fig. 8B). Escalas, 1 µm.*

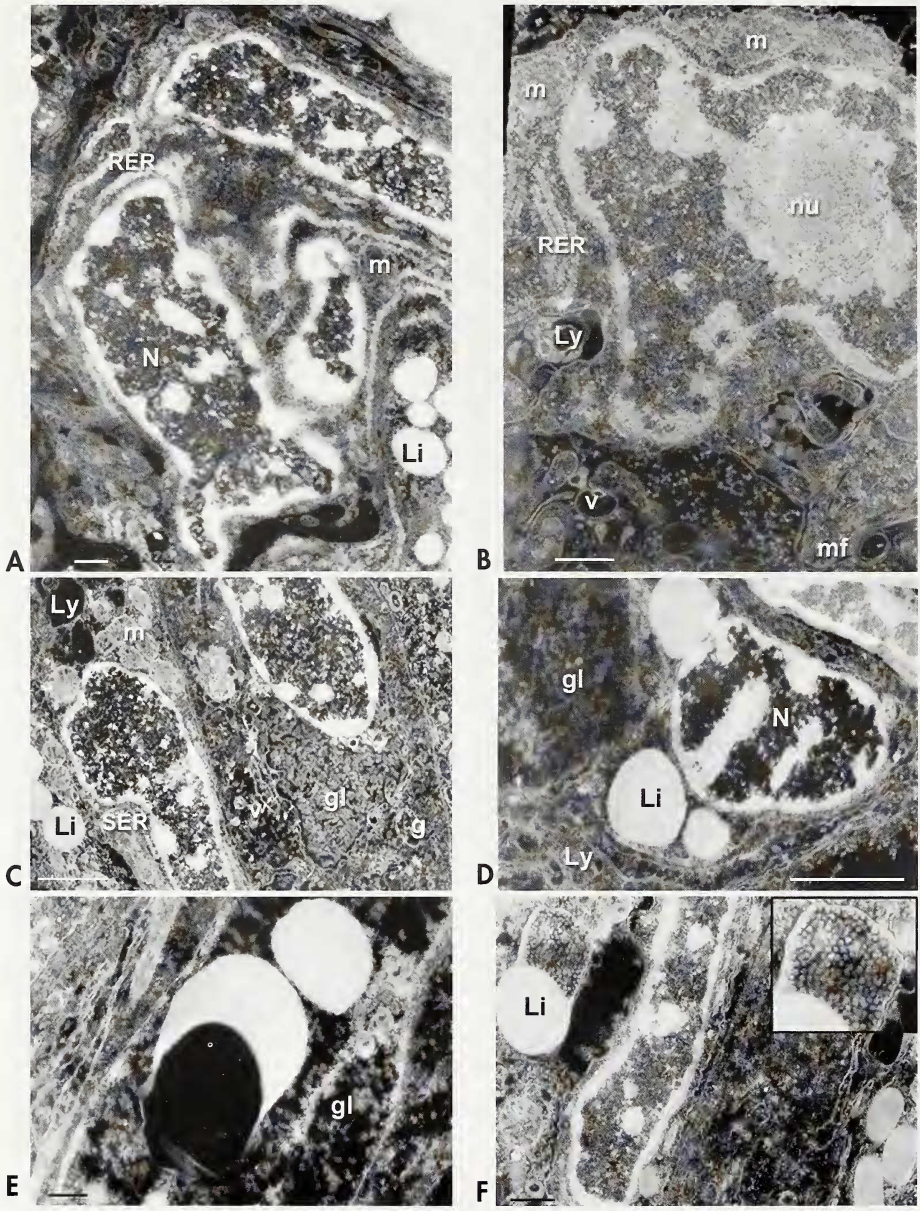

Figure 10. Follicular cells of *Perna perna*. A-F. Irregularly-shaped nuclei. g, granules; gl, particles of glycogen; Li, lipid droplets; Ly, lysosomes; m, mitochondria; mf, myelin-like figures; N, nucleus; nu, nucleolus; RER, cisternae of rough ER; SER, smooth ER. See also lipid droplets in the follicular cells (in D), the presence of granules with two type of electron-lucent materials (in E), and a single inclusion containing a lipid droplet and several glycogen particles forming a rosette (in F). Scale bars, A, B, E: 1 µm; C, D: 5 µm.

Figura 10. Células foliculares de Perna perna. *A-F. Núcleos con forma irregular. g, gránulos; gl, partículas de glucógeno; Li, gotitas de lípidos; Ly, lisosomas; m, mitocóndria; mf, figuras con aspecto de mielina; N, núcleo; nu, nucleolo; RER, cisternas del RE rugoso; SER, RE liso. Nótense también gotitas de lípidos en las células foliculares (en D), la presencia de gránulos con dos clases de materiales electrón lucidos (en E), y una inclusión aislada conteniendo una gotita de lípido y varias partículas de glucógeno formando una roseta (en F). Escalas, A, B, E: 1 µm; C, D: 5 µm.*

Figure 11. Septate desmosome (arrows) between two follicular cells of *Perna perna*. Scale bar, 1 μm.
Figura 11. Desmosoma septado (flechas) entre dos células foliculares de Perna perna. *Escala, 1 μm.*

with no membrane and showing a denser peripheral condensation (Fig. 9B) could be observed. Thirdly, membrane-bound inclusions (Fig. 9C), containing a granular material generally denser at their periphery, resulted from pinocytosis in the periphery of the ooplasm at the end of vitellogenesis. These last granules, which resulted from material extracellular endocytosis contained thin glycogen particles and were different from the cortical granules, described in the late previtellogenic oocytes. Lastly, some granules contained two granular materials, the first being more electron-lucent than the other (Fig. 9D). All these types of inclusions acted in the formation of yolk. Ringed lamellae, probably deriving from the rough ER, surrounded a big yolk granule or several smaller vesicles sometimes with several mitochondria, and developed in the vicinity of lipid droplets (Fig. 7E). They corresponded to Balbiani's vitelline bodies (PIPE, 1987).

In spite of their polyhedral shape, the D oocytes (postvitellogenic oocytes) were deformed by the presence of other oocytes in the lumen of the acinus. The nuclear envelope showed numerous pores and was very indented, while the ring- or crescent-shaped nucleolus was in an eccentric position. Numerous cortical granules, numerous regular yolk vesicles of variable density, and some glycogen deposits linked to lipid droplets (Fig. 9E) were found. In several zones of the cytoplasm, are stacks of rough ER parallel lamellae.

Follicular cells: During previtellogenesis, each oocyte was completely surrounded by small and irregularly-shaped follicular cells (Fig. 10A-D). Each of these last cells contained a big nucleus, of variable form, and projected cytoplasmic extensions into the lateral part of the oocyte. The follicular cells were interconnected together by septate desmosomes (Fig. 11). Their cytoplasm also contained numerous stacks of rough ER cisternae (or annulate lamellae), a smaller zone of smooth ER, numerous mitochondria, well-developed lysosomes, multivesicular bodies, myelin-like figures, and small single or clumped granules of glycogen, sometimes linked to lipid droplets (Fig. 10F). Some other inclusions, each containing a dense material and another more lucent (Fig. 10E), were also present near lipid droplets. The peripheral part of their cytoplasm also contained some pinocytosis vesicles, each containing a lipid droplet and/or a glycogen granule (Fig. 5). When the oocyte was at the B stage of vitellogenesis, the follicular cells became detached from the apex of the oocyte, which bulged freely into the

acinar lumen and became pedunculate, as it was still attached to the inner wall of the acinus. At the C stage of vitellogenesis, the follicular cells were reduced to their basal part and, finally, became completely detached from the oocyte.

In the spaces between the follicular cells and the spindle-shaped cells, free particles of glycogen were frequently observed, particularly during previtellogenesis (Fig. 5).

DISCUSSION

The female gamete formation in *P. perna* was similar to that described in other bivalvia, such as *Brachidontes vigiliae* (BERNARD, DAVIES AND HODGSON, 1988), *Crassostrea virginica* (ECKELBARGER AND DAVIS, 1996), *Mytilus edulis* (ALBERTINI, 1985; PIPE 1987), *Pecten maximus* (DORANGE AND LE PENNEC, 1989), *Pinctada margaritifera* (THIELLEY, 1993), or *Pinna nobilis* (GAULEJAC, HENRY AND VICENTE, 1995). The accumulation of ribosomes and the presence of numerous nuclear pores in previtellogenic oocytes indicated a great synthesis of proteins and an increased transport of material. The perinuclear dense aggregates present in the cytoplasm of these oocytes might correspond to extruded nucleolar material (especially ribonucleoproteins), as described by several authors (ALBERTINI, 1985; DORANGE AND LE PENNEC, 1989; THIELLEY, 1993; GAULEJAC *ET AL.*, 1995). The presence of mitochondria in the stalk of the previtellogenic oocyte suggested a transfer of material. However, the present study did not allow us to observe microtubules in this stalk, such as described in the previtellogenic oocyte of *Pinna nobilis* (GAULEJAC *ET AL.*, 1995), or in that of *Anodonta* (BEAMS AND SEKHON, 1966).

In the vitellogenic oocytes, the growth of cytoplasm in volume was mostly due to the accumulation of inclusions. Among them, the cortical granules were found during all stages of vitellogenesis and this finding agreed with the report by GAULEJAC *ET AL.* (1995) in *Pinna nobilis*. The nature of their contents can only be speculated upon based on morphological investigations. However, evidence for a glycoproteinic content is recognized in oocytes of most bivalve species (e.g. ALBERTINI, 1985; GAULEJAC *ET AL.*, 1995). According to PIPE (1987), the number of these cortical granules might increase via their division. The formation of these cortical granules involved the synthesis of yolk materials by the proteosynthetic organelles of the oocyte. This autosynthetic-type formation has been described in some molluscan species (DE JONG-BRINK, BOER AND JOOSSE, 1983; MEDINA, GARCIA, MORENO AND LOPEZ-CAMPOS, 1986). The production of yolk appears to involve the collaboration of Golgi complexes and RER, as these organelles were observed in close association with yolk bodies, as observed in several mollusc species (ECKELBARGER AND DAVIS, 1996; ECKELBARGER AND YOUNG, 1997). The other types of inclusions found in mature oocytes warrant special comment. First, the two types of lipid droplets, i.e. small inclusions with no membrane and bigger, membrane-bound inclusions, recorded in *P. perna* might correspond to two successive stages in the formation of these lipids via the action of smooth ER, of Golgi apparatus, of mitochondria, and of Balbiani's bodies (DE JONG-BRINK *ET AL.*, 1983). Thus, in the caenogastropod *Colus stimpsoni*, WEST (1983) reported that lipid formation derived from the autosynthetic activities of the oocyte via the endoplasmic reticulum. Secondly, the granules, observed at the C stage of vitellogenesis and containing two types of electron-lucent materials, have not been reported in the literature on bivalvia. To explain this last finding, the most likely hypothesis was to admit that their contents would be of lipoproteinic origin via the fusion of lipid droplets and of proteins originating from rough ER. Lastly, the inclusions resulting from pinocytosis at the end of vitellogenesis were also reported by several authors in other species of molluscs (WEST, 1981, 1983; DE JONG-BRINK *ET AL.*, 1983; ECKELBARGER AND BLADES-ECKELBARGER,

1989; ECKELBARGER AND YOUNG, 1997). Heterosynthetic process involved the Golgi complex and RER on the one hand, and endocytosis of extracellular material on the other hand, so that lipid reserves would be produced by the oocyte and glycogen would be endocytosed (WEST, 1981,1983). In fact, in *P. perna*, the inclusions might originate, either from spindle-shaped cells, which contained glycogen deposits, or from interacinar vesicular cells, which had two types of granules. As free glycogen particles could be seen in the intercellular spaces between the oocyte, the follicular, and the spindle-shaped cells, it might be hypothesized that the free particles would be pinocytosed by the cytoplasm of the vitellogenic oocyte, or by that of follicular cells. Yolk synthesis in *P. perna* was similar to that described in other molluscs species (WEST, 1983; ECKELBARGER AND YOUNG, 1997). However, this massive intake of exogenous substances in the ooplasm was probably not the single way for the formation of yolk. Indeed, the presence of glycogen particles, linked to lipid droplets, in the ooplasm suggested that they might form lipid-carbohydrate complexes, as those described in the oocytes of *Mytilus edulis* (ALBERTINI, 1985). Another way in *P. perna* might be the direct transformation of mitochondria into yolk granules, as reported in the oocytes of many bivalve species (GAULEJAC *ET AL.*, 1995).

The formation of the vitelline membrane occurred during the B and C stages of vitellogenesis, and the material constituting it originated from the oocyte (e.g. PIPE, 1987), the follicular cells (DORANGE AND LE PENNEC, 1989), or both. A part of this material might be composed of the contents of cortical granules, as ALBERTINI (1985) noted the release of mucus from these granules in the vitelline membrane surrounding the oocyte of *Mytilus edulis*. The results noted in the present study indicated the material constituting the vitelline membrane was produced by the oocyte. Our results agreed with those of WOURMS (1987) on the fact that electron microscopy reveals both microvilli and an extracellular coat in the overwhelming majority of invertebrate oocytes.

The ultrastructural changes noted in the atretic oocytes of *P. perna* were similar to those noted in other species of bivalvia, even though the outcome of reserves in *P. perna* was different. The accumulation of yolk granules constituted a dense mass, which is released in the lumen of the acinus, and this oosorption allowed the turn over of nutrients to assure the energetic needs for the bivalve basal metabolism (HOUTTEVILLE, 1974). Another way for re-using oocyte remnants in *P. perna* was constituted by the epithelial cells bordering the gonoducts, as that reported by PIPE (1987) in *Mytilus edulis*, and also by the follicular cells (see the review by DE JONG-BRINK *ET AL.*, 1983).

Some reports were already performed on the relationship between the follicular cells and the maturation of oocytes in bivalvia. The presence of follicular cells completely surrounding the young oocyte and their detachment from it in later stages were already reported in many species (e.g. BERNARD *ET AL.*, 1988; GAULEJAC *ET AL.*, 1995) and this finding underlined the important role of these cells in the growth of oocytes, and their nutrition (DE JONG-BRINK ET AL., 1983). Owing to their organelles, the follicular cells had the capacity to participate in the synthesis of many substances and to phagocytose materials originating from atretic or degenerating oocytes. The exogenous substances, which are intaken by the oocyte of *P. perna* during vitellogenesis via pinocytosis, might originate from the secretions of these follicular cells. The capacity of these last cells to phagocytose the remnants of oocytes in *P. perna* is reflected by the presence of their well-developed lysosomes and of deposits of lipid and glycogen deposits in their cytoplasm. It is possible that products originating from atretic oocytes permitted a transfer of precursors necessary for the vitellogenesis of other developing oocytes, and the synthesis and storage of lipid and glycogen inclusions which can be used later for vitellogenesis, as suggested by GAULEJAC *ET AL.*

(1995) in *Pinna nobilis*. According to WEST (1983), the follicular cells synthesiz-ed glycogen and the oocytes sequestered it through an endocytotic process.

The studies made by ID HALLA *ET AL.* (1997) on the gonad of *P. perna* demonstrated the presence of a single type of reserve cells. However, in the present work, lipid droplets and inclusions of glycogen were present in reserve cells, so that further studies are necessary to determine if different types of reserve cells exist in the gonad of *P. perna* or if the cell found in the present study shows different cellular stages which succeed in relation to the reproductive cycle of *P. perna*.

In conclusion, the formation of female gametes in *P. perna* was greatly similar to the oogenesis described in other species of bivalvia and particularly in Mytilidae. The only difference concerned the composition of the yolk in the mature oocyte, as it was constituted of several endogenous substances (lipoproteins mainly) and also of exogenous materials intaken by the oocyte via pinocytosis. In *P. perna*, vitellogenesis combined both autosynthetic and heterosynthetic processes, and involved the Golgi complex and RER on the one hand, and endocytosis of extracellular material on the other hand. Further studies are necessary to investigate the composition of vitelline granules in the oocytes using cytochemical methods and transmission electron microscopy.

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BIBLIOGRAPHY

ALBERTINI L. 1985. *Recherches cytologiques et expérimentales sur l'ovogénèse chez la moule, (*Mytilus edulis *L., mollusque bivalve)*. Doctoral thesis, University of Caen, 147 p.

BEAMS H.W. AND SEKHON S.S.1966. Electron microscope studies on the oocyte of the fresh-water mussel (*Anodonta*), with special reference to the stalk and mechanism of yolk deposition. *Journal of Morphology*, 119: 477-502.

BENOMAR S., BELHSEN O.K., GOUX D., MATHIEU M. AND MOUKRIM A. 2007. Ultrastructural studies on the spermatogenesis of the African mussel *Perna perna* (Bivalvia: Mytilidae). *Iberus*, 26(2): 1-14.

BERNARD R.T.F., DAVIES B.R. AND HODGSON A.N. 1988. Reproduction in a brackish-water mytilid: gametogenesis and embryonic development. *The Veliger*, 30: 278-290.

BERRY P.F. 1978. Reproduction, growth, and production in the mussel *Perna perna*, on the east coast of South Africa. *Investigational Reports of the Oceanography Research Institute*, Durban, 48: 1-28.

DE JONG-BRINK M., BOER H.H. AND JOOSSE J. 1983. Mollusca. Chapter 14. In: Adiyodi KG, Adiyodi RG, (eds.). Reproductive biology of invertebrates. I. Oogenesis, oviposition, and oosorption, John Wiley and Sons, New York, pp. 297-355.

DORANGE G. AND LE PENNEC M. 1989. Ultrastructural study of oogenesis and oocytic degeneration in *Pecten maximus* from the Bay of St. Brieux. *Marine Biology*, 103: 339-348.

ECKELBARGER K.J. AND BLADES-ECKELBARGER P.I. 1989. Structure of the ovotestis and evidence for hetero synthetic incorporation of yolk precursors in the oocytes of the nudibranch Mollusc, *Spurilla neapolitana*. *Journal of Morphology*, 201: 105-118.

ECKELBARGER K.J. AND DAVIS C.V. 1996. Ultrastructure of the gonad and gametogenesis in the eastern oyster, *Crassostrea virginica*. I. Ovary and oogenesis. *Marine Biology*, 127: 79-87.

ECKELBARGER K.J AND YOUNG C.M. 1997. Ultrastructure of the ovary and oogenesis in the dmethane-seep mollusc *Bathynerita naticoidea* (Gastropoda: Neritidae) from the Louisiana slope. *Invertebrate Biology*, 116 (4): 299-312.

GAULEJAC B. DE, HENRY M. AND VICENTE N. 1995. An ultrastructural study of gametogenesis of the marine bivalve *Pinna nobilis* (Linnaeus 1758). I. Oogenesis. *Journal of Molluscan Studies*, 61: 375-392.

HICKS D.W., TUNNELL J.W. JR AND MCMAHON R.F. 2001a. Population dynamics of the non-indigenous brown mussel, *Perna perna* (Linnaeus 1758), in the Gulf of Mexico compared to other world-wide populations. *Marine Ecology Progress Series*, 211: 181-192.

HICKS D.W., MCMAHON R.F. AND INGRAO D.A. 2001b. Two invasive mussels in the genus *Perna* in the Gulf of Mexico. In: Virtual Proceedings for the State of the Bay Symposium V, 31 January-2 February 2001. Texas Natural Resource Conservation Commission, Austin, Texas, pp. 159-170.

HOUTTEVILLE P. 1974. Contribution à l'étude cytologique et expérimentale du cycle du tissu de réserve du manteau de *Mytilus edulis*. Doctoral thesis, University of Caen.

ID HALLA M., BOUHAIMI A., ZEKHNINI A., NARBONNE J.F., MATHIEU M. AND MOUKRIM A. 1997. Etude du cycle de reproduction de deux espèces de moules *Perna perna* (Linné, 1758) et *Mytilus galloprovincialis* Lamarck, 1819 dans la baie d'Agadir (Sud du Maroc). *Haliotis*, 26: 51-62.

LASIAK T. 1986. The reproductive cycles of the intertidal bivalves *Crassostrea cucullata* (Born, 1778) and *Perna perna* (Linnaeus, 1758) from Transkei coast, Southern Africa. *The Veliger*, 29: 226-230.

MEDINA A., GARCIA J.C., MORENO F.J. AND LOPEZ-CAMPOS J.P. 1986. Comparative studies on the histology of the ovotestis in *Hypselodoris tricolor* and *Godiva banyulensis* (Gastropoda, Opistothobranchia), with special reference to yolk formation. *Journal of Morphology*, 186: 105-118.

PIPE R.K. 1987. Oogenesis in the marine mussel *Mytilus edulis*: an ultrastructural study. *Marine Biology*, 95: 405-414.

SCHURINK C.E. AND GRIFFITHS C.L. 1991. A comparison of reproductive cycle s and reproductive output in four Southern African mussel species. *Marine Ecology Progress Series*, 76: 123-134.

THIELLEY M. 1993. Etude cytologique de la gamétogenèse, de la sex-ratio et du cycle de reproduction chez l'huître perlière *Pinctada margaritifera* (L) var. *cumingii* (Jameson), (Mollusque, bivalves). Comparaison avec le cycle de *Pinctada maculata* (Gould). Doctoral thesis, Université Française du Pacifique, 233p..

WEST D.L. 1981. Reproductive biology of *Colus stimpsoni* (Prosobranchia: Buccinidae). IV. Oogenesis. *The Veliger*, 24: 28-38.

WEST D.L. 1983. Reproductive biology of *Colus stimpsoni* (Prosobranchia: Buccinidae). V. Nutritive egg formation. *The Veliger*, 25: 299-306.

WOURMS J.P. 1987. Oogenesis. In Giese A.C., Pearse J.S. and Pearse V.B. (Eds.): *Reproduction of marine Invertebrates*, Vol. IX, General aspects: seeking unity in diversity: 50-157. Blackwell Scientific Publications and Boxwood Press, Pacific Grove, California.